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(54) Title: METHOD OF SELECTING AT LEAST ONE MUTATION SCREEN, ITS APPLICATION TO A METHOD FOR RAPID IDENTIFICATION OF ALLELES OF POLYMORPHOUS SYSTEMS AND DEVICE FOR IMPLEMENTATION THEREOF

(54) Titre: PROCEDE DE SELECTION D'AU MOINS UN CRIBLE DE MUTATIONS, SON APPLICATION A UN PROCEDE D'IDENTIFICATION RAPIDE D'ALLELES DE SYSTEMES POLYMORPHES ET DISPOSITIF POUR SA MISE EN ŒUVRE

(57) Abstract

Methods of selecting at least one mutation screen from a series of allele sequences of a polymorphous gene and for rapid identification of alleles of polymorphous genes, nucleotide probes obtained from the said mutation screens, placed specifically in data bank form, and a device for implementing the said method are disclosed. The method for identifying alleles consists of: (a) selecting all or part of a known *consensus* sequence of the said polymorphous gene; (b) creating a mutation matrix for the corresponding sequences of known alleles; (c) identifying indiscernable sequences by comparison in twos (alleles having the same mutation profile in the sequence selected in (a)) and excluding one of the members of the said pairs; (d) identifying and counting the obligatory mutations or allele marker mutations, i.e. those which are necessary and adequate for distinguishing between two alleles which are otherwise identical (set O of obligatory mutations); and (f) obtaining the said minimum mutation screen(s), comprising at least the obligatory mutations of step (e); then (g) selecting from the screen selected in step (f) of the said mutation screen selection process the most suitable mutation screen for preparing oligonucleotide probes suitable for use in differenciating all the alleles; (h) appropriately hybridizing an allele X to be identified with the oligonucleotide probes selected from the mutation screen(s) obtained in steps (a) to (g); and (i) identifying the allele X by detection of the said hybrid(s) which may have been formed in step (h).

VERIFIED TRANSLATION OF PCT 33550/93

VERFICATION OF TRANSLATION

RE: INTERNATIONAL APPLICATION NO. PCT/FR92/01141

I, Abraham SMITH, Dip.Ing., Dip.Doc.,
translator to RWS Translations Ltd., of Europa House, Marsham Way, Gerrards Cross, Buckinghamshire, England, am the translator of the documents attached and I state that the following is a true translation to the best of my knowledge and belief.

Signature of translator Abra Smith

For and on behalf of RWS Translations Ltd.

Dated: 20 May 1994

Method of selecting at least one mutation screen, its application to a method for the rapid identification of alleles of polymorphous systems and device for implementation thereof.

The present invention relates to a method of selecting at least one mutation screen from a set of allelic sequences of a polymorphous gene, to a method for the rapid identification of allelic variations (alleles or allelic sequences) of the sequences of polymorphous genes, to nucleotide probes obtained from the said mutation screens, especially placed in the form of data banks, as well as to a device for implementing the said methods.

The present invention also relates to a kit for the identification of the alleles of polymorphous genes.

At present, it is very difficult and very tedious to identify the different alleles of the same gene, differing by mutation of at least one base in their nucleotide sequence, especially in the case of naturally polyallelic systems, such as the major histocompatibility system (HLA) whose genes can exist in numerous allelic forms, as well as in any other form of polymorphism, especially those due to somatic mutations such as those of immunoglobulins and T cell receptors or alternatively those encountered in systems equivalent to a polyallelic system, which are more particularly observed in certain multiple-mutation genetic diseases such as cystic fibrosis or Duchenne's muscular dystrophy.

The major histocompatibility complex (HLA complex) genes, for example, are closely linked on the short arm of chromosome 6 and extend over about 5000 kb; they encode three types of proteins, the class I, II and III proteins; a major characteristic of the HLA system is its vast polymorphism. The polymorphism of this system results from the number of genes and the number of different alleles which are possible for each of these

genes, the polymorphism being further increased if the fact that an individual may have received the same allele from both its parents (homozygous state) or may have received two different alleles (heterozygous state) is taken into account.

Furthermore, if it is considered that for the HLA complex, there may be from 10 to 100 alleles per gene and that 15 to 20 genes encoding the proteins of the HLA complex have currently been characterized, it is practically impossible to carry out a complete typing (or identification) of this complex with the methods currently available, whereas the latter may prove crucial, especially in transplantation.

Indeed, the typing of the different polymorphous systems can be currently performed either by immunochemical methods or by DNA/DNA hybridization techniques; however, these techniques have the disadvantage:

- of not being sufficiently discriminatory, and therefore of not permitting the differentiation of alleles of very similar structures and
- of necessitating the use of a large number of oligonucleotide probes (for example: about 50-60 probes in the case of the DRß gene of the HLA system (see especially the nomenclature of the factors of the HLA system, published in 1990 in Immunogenetics, 31, 131-140), which comprises 56 alleles), and this, insofar as in the molecular biology-based conventional typing methods of the prior art, it is effectively necessary to provide of the order of one probe per allele in order to be able to interpret the result.

Now, this identification is often necessary either for preventive reasons, or for curative reasons (especially therapy, surgery, transplants); more particularly in the case of the HLA complex, the control of a reliable typing system is made necessary for a preventive purpose by the existence of a correlation between the susceptibility to certain diseases and the

frequency of certain HLA alleles; and for a curative purpose by the necessity to have an HLA compatibility between donor and recipient, in the case of a transplant, as specified above and for the purpose of identifying individuals (especially criminology and search for paternity).

Consequently, the Applicant set itself the objective of providing a method for rapid and reliable identification of alleles, which has the advantage of permitting the identification of the complete allelic map of a subject, and this without necessitating the use of a large number of oligonucleotide probes (difficulty of production and high cost of the said probes).

The subject of the present invention is a method for the selection, from a series of allelic sequences of a polymorphous gene, of at least one mutation screen intended to specify at least one nucleotide probe suitable for use in differentiating all the alleles, characterized in that it comprises the following steps:

(a) selecting all or part of a known *consensus* sequence of the said polymorphous gene;

(b) creating a mutation matrix for the corresponding sequences of known alleles;

(c) identifying indiscernible sequences by comparison in twos (alleles having the same mutation profile in the sequence selected in (a)) and excluding one of the members of the said pairs;

(e) identifying and counting the obligatory mutations or so-called allele marker mutations, that is to say those which are necessary and adqueate for distinguishing between two alleles which are otherwise identical (set O of obligatory mutations); and

(f) obtaining the said minimum mutation screen(s), comprising at least the obligatory mutations of step (e).

According to an advantageous embodiment of the said method, prior to step (e) for identification and counting of the obligatory mutations, the said method

comprises:

(d) identifying similar mutations in each of the said sequences of alleles of step (b), so as to treat in the next steps only the mutations which are non-redundant and which constitute the set U of useful mutations; which step (d) is followed by the steps (e) and (f) modified as follows:

(e) identifying and counting the obligatory mutations or so-called allele marker mutations, among the useful mutations of the set U, that is to say those which are necessary and adequate for distinguishing between two alleles which are otherwise identical (set O' of obligatory mutations); and

(f) if the obligatory mutations of step (e) do not permit mutation screens suitable for the univocal differentiation of all the alleles to be directly obtained, a minimum number of useful mutations of step (d) (subset U_1 derived from the set U of useful mutations) is selected, which mutations, associated with the obligatory mutations of step (e), form the mutation screen(s) suitable for the univocal differentiation of all the alleles.

According to an advantageous arrangement of this embodiment, prior to step (f), the said method comprises a step (x) for selecting useful mutations of step (d) (subset U_2 derived from the set U of useful mutations), in order to form a group of useful mutations most suitable for preparing oligonucleotide probes suitable for use in differentiating all the alleles; which step (x) is followed by the step (f) modified as follows:

(f) if the obligatory mutations do not permit the direct selection of mutation screens suitable for the univocal differentiation of all the alleles, a minimum number of useful mutations of step (x) is selected, which mutations, associated with the obligatory mutations of step (e), form the mutation screen(s) suitable for the univocal differentiation of all the alleles.

Such mutation screens are particularly

advantageous for the selection and preparation of a limited number of oligonucleotide probes suitable for use in differentiating all the alleles of a polymorphous gene.

The subject of the present invention is also a method for the identification of alleles (or allelic sequences) of a polymorphous gene, characterized in that it comprises the following steps:

I - selecting at least one mutation screen prepared from a series of allelic sequences of a polymorphous gene in the steps:

. (a) to (f) of the method of selecting at least one mutation screen as defined above (including the different variants); then

. (g) choosing, from the screens selected in step (f) of the said mutation screen selection process, the most suitable mutation screen for selecting and preparing oligonucleotide probes suitable for use in differentiating all the alleles;

II - actual typing of an allele X to be identified by:

(h) appropriately hybridizing the said allele X with the oligonucleotide probes selected from the mutation screen(s) obtained in steps (a) to (g); and

(i) identifying the allele X by detection of the said hybrid(s) which may have been formed in step (h).

Advantageously, when the mutation screen selection process comprises step (d) as defined above, the said step (d) has the advantage of bringing about a first reduction in the mutations to be considered in the subsequent steps, by eliminating a first subset of mutations (redundant mutations) and therefore of constituting a set U of mutations useful for the characterization of an allele.

The steps (e) to (g) have the advantage:

- of permitting the selection of a subset of obligatory mutations, among the useful mutations of the set U which, optionally in association with:

- either a subset U_1, derived from the set U of useful mutations (the set U_1 corresponding to a minimum number of useful mutations which, in association with the obligatory mutations, form mutation screens suitable for the univocal differentiation of all the alleles)

- or a subset U_2, derived from the set U of useful mutations and selected in order to form a group of useful mutations more suitable for preparing appropriate oligonucleotide probes, form mutation screens suitable for the univocal differentiation of all the alleles; and

- of permitting, because of the selection of the specific oligonucleotide probes, a rapid identification of the unknown allele.

Indeed, the method conforming to the invention permits, in addition to the selection of a limited number of oligonucleotide probes, the selection of probes having the following advantageous characteristics:

- maximum pairing with the *consensus* sequence;
- absence of formation of sequences giving rise to the formation of non-specific homo- or heterodimers;
- high content of GC bases; and
- absence of polypurine or polypyrimidine repetitive sequences.

Furthermore, the method conforming to the invention permits the direct identification of homozygous doublets and their differentiation from heterozygous doublets.

In this latter case, in order to obtain, *in fine*, the mutation screen, the same method as described above is used comprising the analysis of each sequence of the doublet at each position; it is therefore the doublets of alleles which are compared with all the other doublets of alleles.

The preventive and curative implications of the precise knowledge of the alleles carried by a given subject are important; the method conforming to the invention makes it possible, in a very short time, to solve this problem.

The subject of the present invention is also the application of the method for the selection of at least one mutation screen from a series of allelic sequences of a polymorphous gene, to the preparation of a data bank consisting of a series of mutation screens obtained by the above method and intended for the preparation of oligonucleotide probes suitable for use in differentiating all the alleles.

The subject of the present invention is also oligonucleotide probes, characterized in that they are constructed for the use of at least one mutation screen derived from the method of selection of at least one mutation screen from a series of allelic sequences of a polymorphous gene or from the data bank as defined above, in that they comprise between 15 and 50 bases and in that they are the most suitable for hybridizing with an allelic sequence for the identification of alleles of a polymorphous gene.

Such probes can be optionally labelled by means of a marker such as a radioactive isotope, an appropriate enzyme, a fluorochrome, an antibody or a base analogue; such probes can also be constructed for use in the method for detecting and/or identifying a specific nucleotide base present in a nucleic acid sequence (mutation) described in European Patent Application 412 883, in the Applicant's name.

According to an advantageous embodiment of the said probes, they comprise a sequence derived from the selected *consensus* sequence and whose nucleotide base situated at the 3' end corresponds to a base upstream of one of the mutant bases of the selected mutation screen.

The subject of the present invention is also a kit for the identification of alleles of a polymorphous gene, characterized in that it comprises at least:

- appropriate quantities of a collection of oligonucleotide probes conforming to the invention; optionally associated with:
- appropriate quantities of a reagent for

detection of the probe-sequence to be identified hybrids possibly formed; and/or with

- a table for interpretation of the result of the hybridizations obtained, as a function of the selected mutation screen.

According to an advantageous embodiment of the said kit, the said probes comprise a sequence derived from the selected *consensus* sequence and whose nucleotide base situated at the 3′ end corresponds to a base upstream of one of the mutant bases of the selected mutation screen.

According to another advantageous embodiment of the said kit, it additionally comprises:

- appropriate quantities of four nucleotide bases modified so as to be incorporable into the product of extension of the said probes used as primers, while blocking the elongation of the said extension product.

Such an embodiment permits the use of the method described in European Patent Application 412 883 in the Applicant's name.

The subject of the present invention is, in addition, a device for implementing the method conforming to the invention, characterized in that it comprises at least:

- means for input of data,
- means for programmed calculation in order to generate the mutation screen(s),
- means for storing the said screens, and
- means capable of permitting the identification of the alleles from the stored screens.

In addition to the preceding arrangements, the invention also comprises other arrangements which will become apparent from the following description, which refers to exemplary embodiments of the method which is the subject of the present invention as well as to the accompanying drawing, in which:

- Figure 1 illustrates an embodiment of the method of selecting a mutation screen in which the said

screen is directly obtained from the set O of obligatory mutations;

- Figure 2 illustrates another embodiment in which the said screen is obtained from a set O' of obligatory mutations derived from a set U of useful mutations, which set O' is optionally associated with a subset U_1 or with a subset U_2 of useful mutations, as defined above;

- Figure 3 illustrates a device for implementing the methods conforming to the invention (creation phase and exploitation phase);

- Figure 4 illustrates a mutation matrix for a sequence of 7 alleles, called All;

- Figure 5 illustrates the set U (useful mutations) for identifying a pair of homozygous alleles of the All sequence;

- Figure 6 illustrates the mutation screens suitable for the univocal identification of all the pairs of homozygous alleles of All;

- Figure 7 illustrates the set U (useful mutations) for identifying a pair of heterozygous alleles of the All gene;

- Figure 8 illustrates the mutation screen suitable for the univocal identification of all the doublets of heterozygous alleles of All;

- Figure 9 illustrates the set U (useful mutations) for identifying a pair of homozygous alleles of the DQß 1 gene; and

- Figure 10 illustrates the set of mutation screens suitable for the identification of all the pairs of homozygous alleles of the DQß 1 gene.

It should be understood, however, that these examples are given solely by way of illustration of the subject of the invention and do not constitute in any manner a limitation thereof.

A device conforming to the invention permits the implementation of the methods of selection and identification as defined above both in the creation

phase (constitution of the screens) and in the exploitation phase (identification of an allele).

In the creation phase, the mutation matrix for alleles is introduced in (1) into an appropriate microprocessor (A) and generates in (4), by means of the method for selecting at least one mutation screen conforming to the invention, a set of screens, which are stored in (3, 3′) in a data bank.

In the exploitation phase, a sequence to be identified is hybridized with a collection of suitable probes, constructed for the use of at least one mutation screen; from the hybrids obtained, the sequence is identified (experimental data introduced in (2)); the result obtained is compared with the screen in (5), which makes it possible to specify the allele in question.

EXAMPLE 1: Constitution of mutation screens for the homozygous alleles of the All gene.

. the sequence All*0501 is selected as *consensus* sequence as seen in Figure 4, in which the first sequence is considered as *consensus* sequence; in the other sequences, only the mutations with respect to the said *consensus* sequence are indicated.

. the alleles are compared in twos and the mutations useful for differentiating each pair of alleles are identified: the useful mutations found with the method conforming to the invention are 9 in number:
5, 8, 14, 19, 20, 21, 36, 48, 49
in conformity with Figure 5.
In this example, a pair of alleles is indiscernible (pair All*0201 and All*0202); the allele All*0202 is consequently suppressed for the rest of the analysis.

. the search for the obligatory mutations is then carried out:
All*0401 and All*0501 differ by only 36.

It emerges from this research that only one obligatory position exists among the useful mutations, it is position 36.

. in the present case, the only obligatory

mutation does not permit all the possible pairs of alleles to be differentiated; no "solutions" exist, that is to say screens which permit the univocal identification of all the alleles considered, with a number of mutations of less than 3 (that is to say 2 additional mutations). All the possible mutation screens with 3 mutations are:

1) 36, 5, 20
2) 36, 8, 20
3) 36, 14, 20,

in conformity with Figures 6.1 to 6.3 and show that it is possible to identify an allele of the All gene with the aid of any one of these mutation screens.

EXAMPLE 2: Constitution of mutation screens for the heterozygous alleles of the All gene.

In this example, after execution of the steps as described in Example 1, and which result in the identification of the useful mutations as seen in Figure 7, the search is carried out for the obligatory mutations which permit differentiation of all the doublets of alleles:

All*0401, All*0401 and All*0501, All*0401 differ by only 36;
All*0401, All*0401 and All*0501, All*0501 differ by only 36;
All*0302, All*0401 and All*0501, All*0302 differ by only 36;
All*0301, All*0401 and All*0501, All*0301 differ by only 36;
All*0201, All*0401 and All*0501, All*0201 differ by only 36;
All*0502, All*0401 and All*0501, All*0502 differ by only 36;
All*0501, All*0401 and All*0501, All*0501 differ by only 36.

It emerges from this search that only one obligatory position exists among the useful mutations; it is position 36.

In this example, this single obligatory mutation does not permit differentiation of all the doublets of alleles. No "solutions" exist with a number less than 3, that is to say 2 additional positions. One of the mutation screens which permits differentiation of all the doublets of alleles is: 36, 5, 20, in conformity with Figure 8.

EXAMPLE 3: Typing of an All heterozygous individual.

The mutation screen of Example 2 is chosen in order to identify alleles, because it is the most suitable for the preparation of probes which correspond to the selection criteria defined above (maximum pairing with the *consensus* sequence, absence of sequences giving rise to the formation of homo- or heterodimers, high content of GC bases and absence of polypurine or polypyrimidine repetitive sequences).

Probes of 20 oligonucleotides are synthesized such that position 3′ of the said probes corresponds to a base situated just upstream of one of the positions of the above screens, such that when the hybridization and extension under the conditions of the abovementioned European Patent Application is carried out, it is possible to verify which of the base(s) hybridize(s). The use of such a panel of probes makes it possible to identify

- in individual 1, the sequence CC CT CC which, with reference to the chosen screen, makes it possible to identify the pair of alleles All*0201, All*0302, and
- in the tested individual 2, the sequence CC CT CG which, with reference to the chosen screen, makes it possible to identify the pair of alleles All*0502, All*0201.

EXAMPLE 4: Constitution of mutation screens for the homozygous alleles of the HLA-DQß 1 gene

The nomenclature of the factors of the HLA system was published in 1990 in Immunogenetics, 31, 131-140 and the following example illustrates the constitution of a mutation screen for the alleles of the HLA-DQß 1 gene, as

defined in this article.

. the sequence DQß 1*0501 (position 1 to position 300) is selected as *consensus* sequence;

. the positions of similar mutations are identified so that they are considered only once; the following result is obtained:

* mutation 25 is similar to 7;
* mutation 140 is similar to 110;
* mutation 186 is similar to 167;
* mutation 266 is similar to 250;
* mutation 269 is similar to 259;
* mutation 280 is similar to 277;

consequently, mutations 25, 140, 186, 266, 269 and 280 are ignored in the next step of the method (the numbers correspond to the positions of the mutations on the sequence).

. the alleles are compared in twos and the mutations useful for differentiating each pair of alleles are identified: the useful mutations found with the method conforming to the invention are 54 in number:
7 26 38 40 57 63 68 75 76 77 81 83 88 89 105 109 110 113 114 134 137 141 144 147 153 155 158 164 167 169 170 171 198 199 208 209 211 212 213 216 220 221 223 230 231 234 250 253 257 259 260 265 271 277, in conformity with Figure 9.

In this example, all the pairs of alleles can be differentiated.

. the search for the obligatory mutations is then carried out:
DQß 1*0402 and DQß 1*0401 differ by only 68,
DQß 1*03032 and DQß 1*03031 differ by only 63,
DQß 1*03032 and DQß 1*0302 differ by only 170.

It emerges from this search that the obligatory positions among the useful mutations are 63, 68 and 170.

. in the present case, the three obligatory mutations do not permit differentiation of all the possible pairs of alleles; no solutions exist with a number of mutations of less than 7 (that is to say 4

additional mutations). All the possible mutation screens with 7 mutations are:

1- 63, 68, 170, 7, 76, 88, 171
2- 63, 68, 170, 7, 77, 88, 171
3- 63, 68, 170, 26, 76, 88, 171,
4- 63, 68, 170, 26, 76, 88, 231,
5- 63, 68, 170, 26, 77, 88, 171,
6- 63, 68, 170, 26, 77, 88, 231,
7- 63, 68, 170, 57, 76, 88, 171,
8- 63, 68, 170, 57, 77, 88, 171,
9- 63, 68, 170, 76, 88, 109, 171,
10- 63, 68, 170, 76, 88, 113, 171,
11- 63, 68, 170, 76, 88, 114, 171,
12- 63, 68, 170, 76, 88, 114, 231,
13- 63, 68, 170, 76, 88, 134, 171,
14- 63, 68, 170, 76, 88, 141, 171,
15- 63, 68, 170, 76, 88, 141, 231,
16- 63, 68, 170, 76, 88, 153, 171,
17- 63, 68, 170, 76, 88, 158, 171,
18- 63, 68, 170, 76, 88, 158, 231,
19- 63, 68, 170, 76, 88, 164, 171,

and

20- 63, 68, 170, 76, 88, 164, 231,

in conformity with Figures 10.1 to 10.20 (in which the allele DQß 1 is represented by DQßB1) and show that it is possible to identify an allele of the DQß 1 gene with the aid of any one of these mutation screens.

As evident from the above, the invention is not in any way limited to those of its embodiments, implementations and applications which have just been described more explicity; it embraces, on the contrary, all the variants which may occur to the specialist in this field, without departing from the framework or the scope of the present invention.

CLAIMS

1. Method for the selection, from a series of allelic sequences of a polymorphous gene, of at least one mutation screen intended to specify at least one nucleotide probe suitable for use in differentiating all the alleles, characterized in that it comprises the following steps:

(a) selecting all or part of a known *consensus* sequence of the said polymorphous gene;

(b) creating a mutation matrix for the corresponding sequences of known alleles;

(c) identifying indiscernible sequences by comparison in twos (alleles having the same mutation profile in the sequence selected in (a)) and excluding one of the members of the said pairs;

(e) identifying and counting the obligatory mutations or so-called allele marker mutations, that is to say those which are necessary and adqueate for distinguishing between two alleles which are otherwise identical (set O of obligatory mutations); and

(f) obtaining the said minimum mutation screen(s), comprising at least the obligatory mutations of step (e).

2. Method of selection according to Claim 1, characterized in that, prior to step (e) for identification and counting of the obligatory mutations, the said method comprises:

(d) identifying similar mutations in each of the said sequences of alleles of step (b), so as to treat in the next steps only the mutations which are non-redundant and which constitute the set U of useful mutations; which step (d) is followed by the steps (e) and (f) modified as follows:

(e) identifying and counting the obligatory mutations or so-called allele marker mutations, among the useful mutations of the set U, that is to say those which are necessary and adequate for distinguishing between two

alleles which are otherwise identical (set O' of obligatory mutations); and

(f) if the obligatory mutations of step (e) do not permit mutation screens suitable for the univocal differentiation of all the alleles to be directly obtained, a minimum number of useful mutations of step (d) (subset U_1 derived from the set U of useful mutations) is selected, which mutations, associated with the obligatory mutations of step (e), form the mutation screen(s) suitable for the univocal differentiation of all the alleles.

3. Method of selection according to Claim 2, characterized in that, prior to step (f), the said method comprises a step (x) for selecting useful mutations of step (d) (subset U_2 derived from the set U of useful mutations), in order to form a group of useful mutations most suitable for preparing oligonucleotide probes suitable for use in differentiating all the alleles; which step (x) is followed by the step (f) modified as follows:

(f) if the obligatory mutations do not permit the direct selection of mutation screens suitable for the univocal differentiation of all the alleles, a minimum number of useful mutations of step (x) is selected, which mutations, associated with the obligatory mutations of step (e), form the mutation screen(s) suitable for the univocal differentiation of all the alleles.

4. Method for the identification of alleles of a polymorphous gene, characterized in that it comprises the following steps:

I - selecting at least one mutation screen prepared from a series of allelic sequences of a polymorphous gene in the steps:

. (a) to (f) of the method of selection according to any one of Claims 1 to 3; then

. (g) choosing, from the screens selected in step (f) of the said mutation screen selection process, the most suitable mutation screen for preparing

oligonucleotide probes suitable for use in differentiating all the alleles;

II - actual typing of an allele X to be identified by:

(h) appropriately hybridizing the said allele X with the oligonucleotide probes selected from the mutation screen(s) obtained in steps (a) to (g); and

(i) identifying the allele X by detection of the said hybrid(s) which may have been formed in step (h).

5. Application of the method according to any one of Claims 1 to 3, to the preparation of a data bank consisting of a series of mutation screens obtained by the above method and intended for the preparation of oligonucleotide probes suitable for use in differentiating all the alleles.

6. Oligonucleotide probes, characterized in that they are constructed for the use of at least one mutation screen derived from the method of selection according to any one of Claims 1 to 3 or from the data bank prepared according to Claim 5, in that they comprise between 15 and 50 bases and in that they are the most suitable for hybridizing with an allelic sequence for the identification of alleles of a polymorphous gene.

7. Probes according to Claim 6, characterized in that they comprise a sequence derived from the selected *consensus* sequence and whose nucleotide base situated at the 3' end corresponds to a base upstream of one of the mutant bases of the selected mutation screen.

8. Kit for the identification of alleles of a polymorphous gene, characterized in that it comprises at least:

- appropriate quantities of a collection of oligonucleotide probes according to Claim 6 or Claim 7; possibly associated with:

- appropriate quantities of a reagent for detection of the probe-sequence to be identified hybrids possibly formed; and/or with

- a table for interpretation of the result of the

hybridizations obtained, as a function of the selected mutation screen.

9. Kit according to Claim 8, characterized in that the said probes comprise a sequence derived from the selected *consensus* sequence and whose nucleotide base situated at the 3′ end corresponds to a base upstream of one of the mutant bases of the selected mutation screen.

10. Kit according to Claim 8 or Claim 9, characterized in that it additionally comprises:

- appropriate quantities of four nucleotide bases modified so as to be incorporable into the product of extension of the said probes used as primers, while blocking the elongation of the said extension product.

11. Device for implementing the methods according to any one of Claims 1 to 4, characterized in that it comprises at least:

- means for input of data (1, 2),
- means for programmed calculation in order to generate the mutation screen(s) (4),
- means for storing the said screens (3, 3′), and
- means (5) capable of permitting the identification of the alleles from the stored screens.

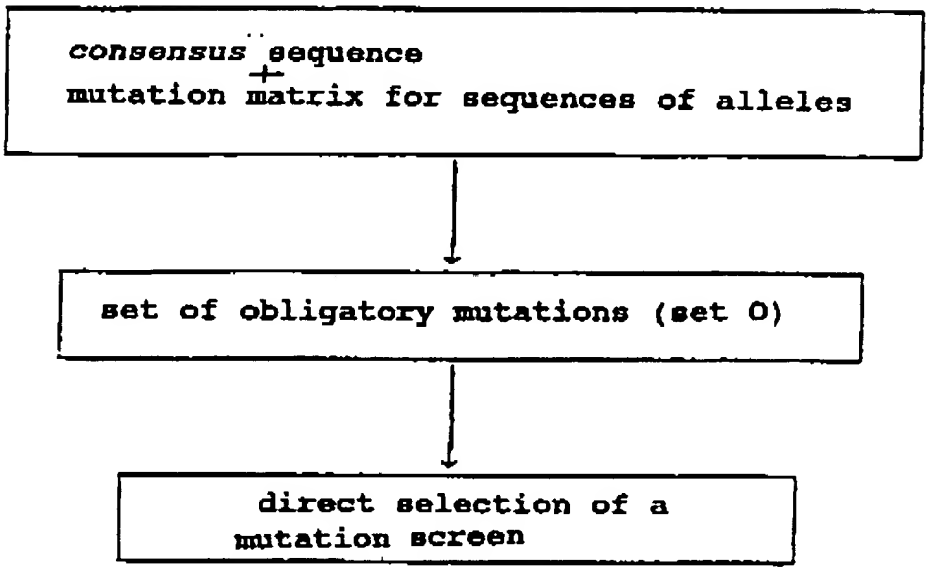
consensus sequence
+
mutation matrix for sequences of alleles
set of obligatory mutations (set O)
direct selection of a
mutation screen

FIGURE 1

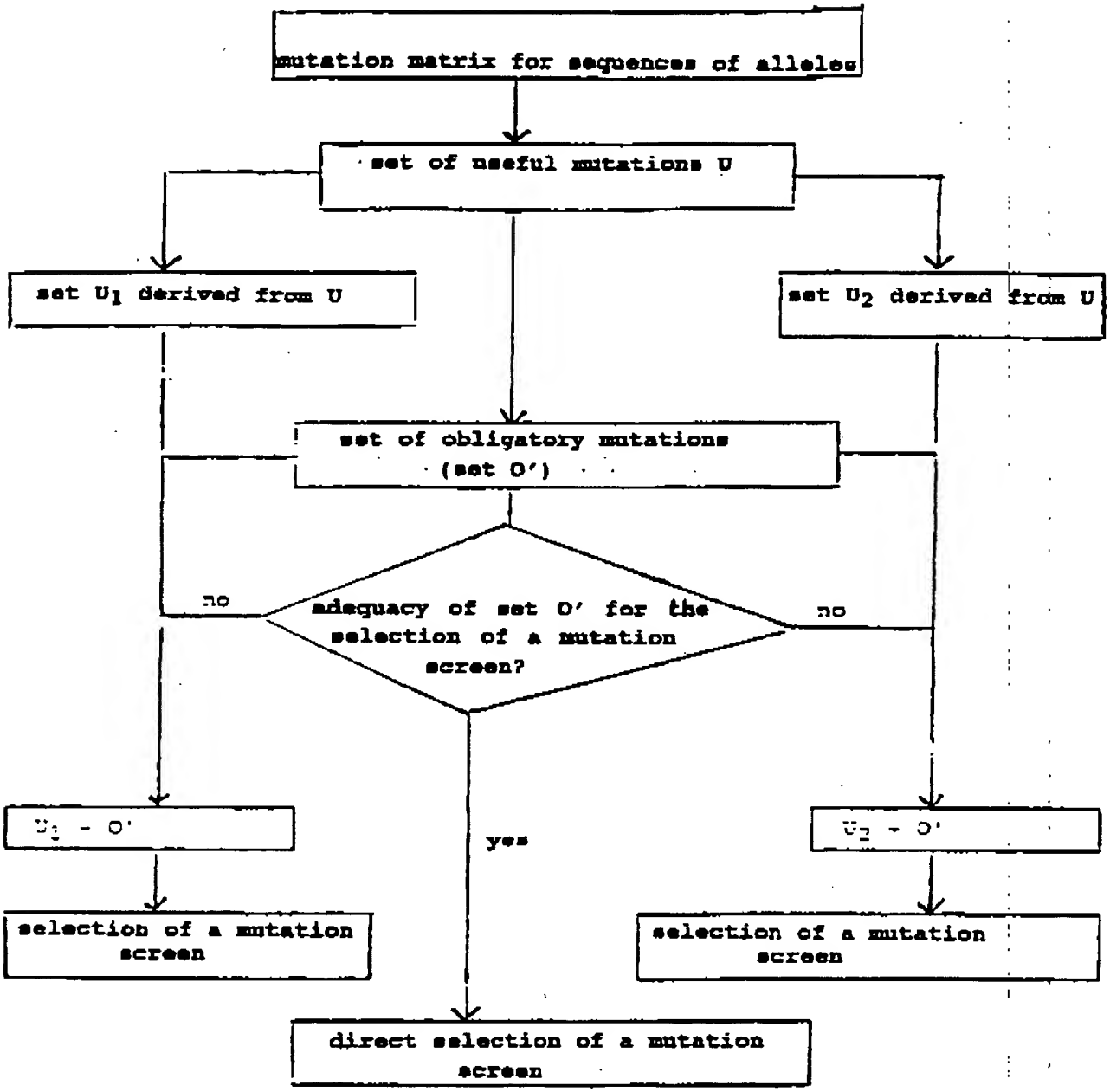
mutation matrix for sequences of alleles
set of useful mutations U
set U_1 derived from U
set U_2 derived from U
set of obligatory mutations (set O')
adequacy of set O' for the selection of a mutation screen?
no
no
yes
U_1 - O'
U_2 - O'
selection of a mutation screen
selection of a mutation screen
direct selection of a mutation screen

FIGURE 2

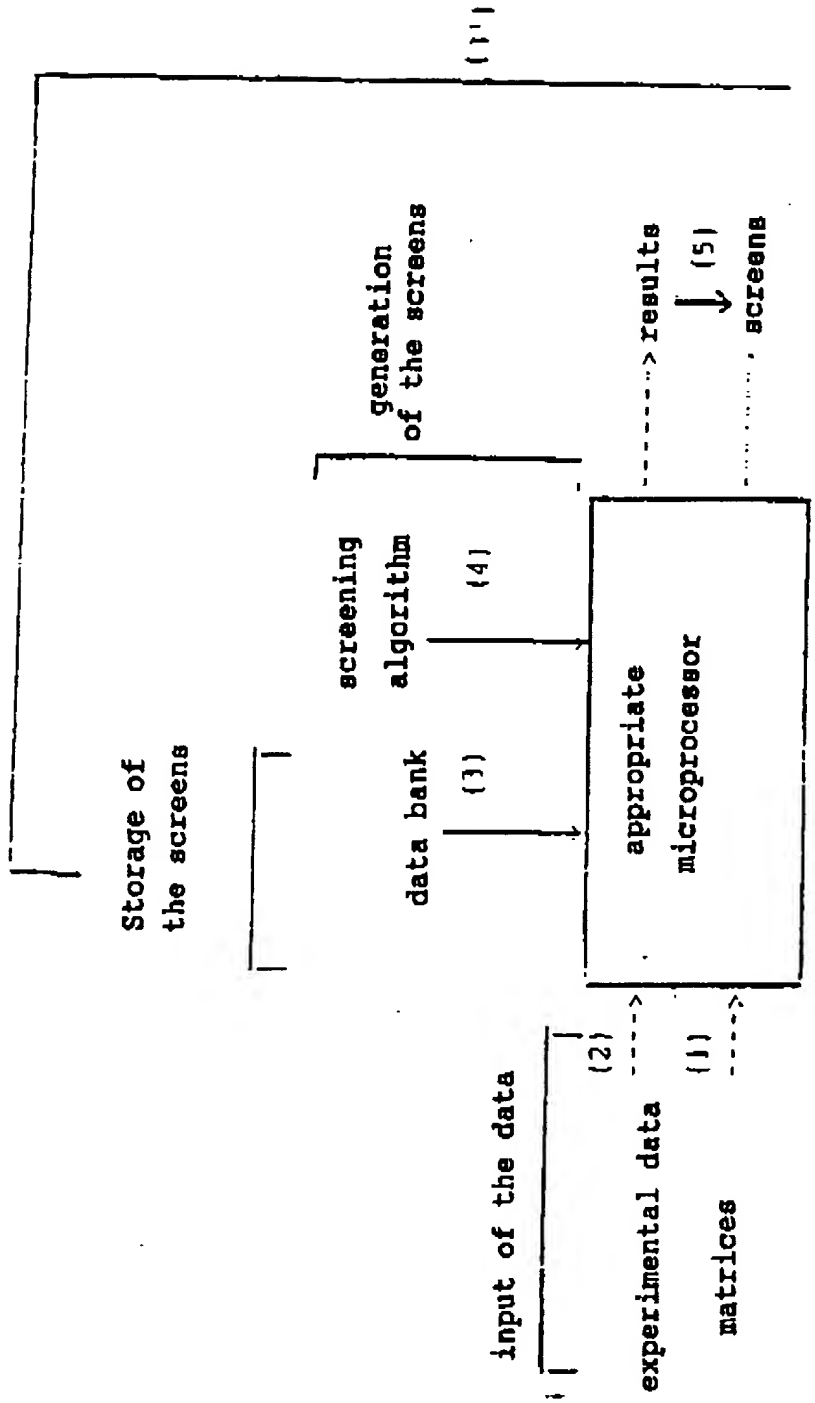
(11)
generation of the screens
screening algorithm
(4)
data bank
(3)
Storage of the screens
appropriate microprocessor
results
(5)
screens
(2)
(1)
input of the data
experimental data
matrices

FIGURE 3

FIGURE 4

FIGURE 5

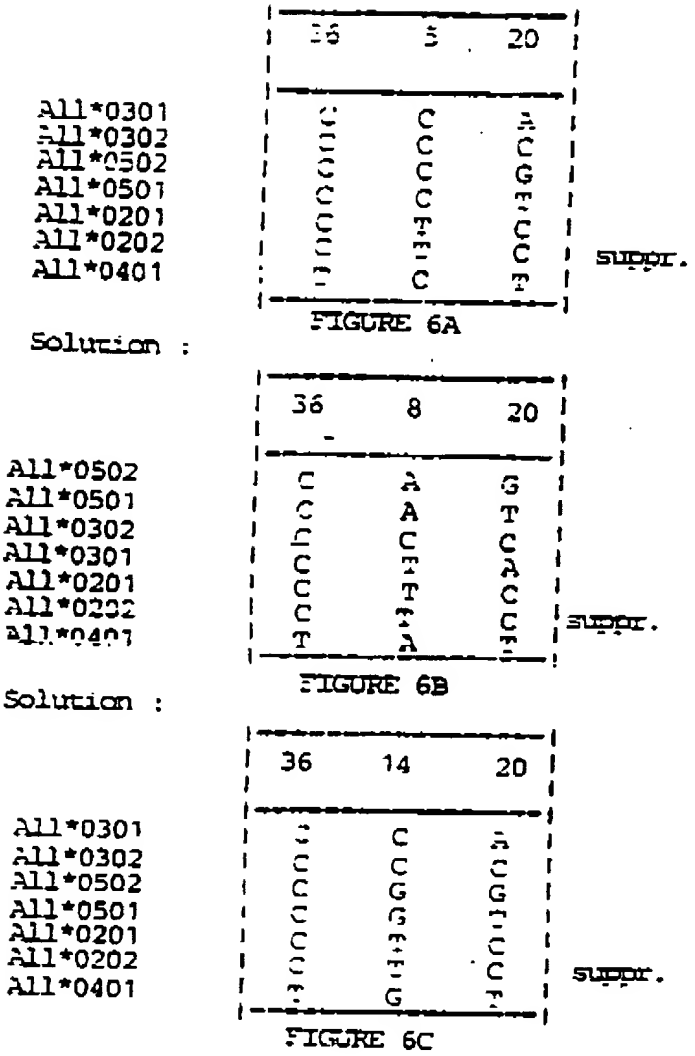

FIGURE 6A

FIGURE 6B

FIGURE 6C

FIGURE 6

	5	8	14	19	20	21	36	48	49
A11*0501,A11*0501	G	A	G	G	T	T	G	A	G
A11*0501,A11*0502	G	A	G	A	T	T	G	A	A
A11*0501,A11*0201	G	A	G	A	T	T	G	A	A
A11*0501,A11*0301	G	A	G	A	T	T	G	A	A
A11*0501,A11*0302	G	A	G	A	T	T	G	A	A
A11*0501,A11*0401	G	A	G	A	T	T	G	A	A
A11*0502,A11*0502	G	A	G	A	T	T	G	A	A
A11*0502,A11*0201	G	A	G	A	T	T	G	A	A
A11*0502,A11*0301	G	A	G	A	T	T	G	A	A
A11*0502,A11*0302	G	A	G	A	T	T	G	A	A
A11*0502,A11*0401	G	A	G	A	T	T	G	A	A
A11*0201,A11*0201	G	A	G	A	T	T	G	A	A
A11*0201,A11*0301	G	A	G	A	T	T	G	A	A
A11*0201,A11*0302	G	A	G	A	T	T	G	A	A
A11*0201,A11*0401	G	A	G	A	T	T	G	A	A
A11*0301,A11*0301	G	A	G	A	T	T	G	A	A
A11*0301,A11*0302	G	A	G	A	T	T	G	A	A
A11*0301,A11*0401	G	A	G	A	T	T	G	A	A
A11*0302,A11*0302	G	A	G	A	T	T	G	A	A
A11*0302,A11*0401	G	A	G	A	T	T	G	A	A
A11*0401,A11*0401	G	A	G	A	T	T	G	A	A

FIGURE 1

	36	5	20
A11*0301,A11*0301	G	G	A
A11*0301,A11*0302	G	G	A
A11*0502,A11*0301	G	G	A
A11*0501,A11*0301	G	G	A
A11*0302,A11*0302	G	G	A
A11*0502,A11*0302	G	G	A
A11*0501,A11*0302	G	G	A
A11*0502,A11*0502	G	G	G
A11*0501,A11*0502	G	G	G
A11*0501,A11*0501	G	G	G
A11*0201,A11*0301	G	G	T
A11*0201,A11*0302	G	G	T
A11*0502,A11*0201	G	G	A
A11*0501,A11*0201	G	G	A
A11*0201,A11*0201	G	G	A
A11*0301,A11*0401	G	G	A
A11*0302,A11*0401	G	G	A
A11*0502,A11*0401	G	G	A
A11*0501,A11*0401	G	G	A
A11*0201,A11*0401	G	G	A
A11*0401,A11*0401	G	G	A

FIGURE 2

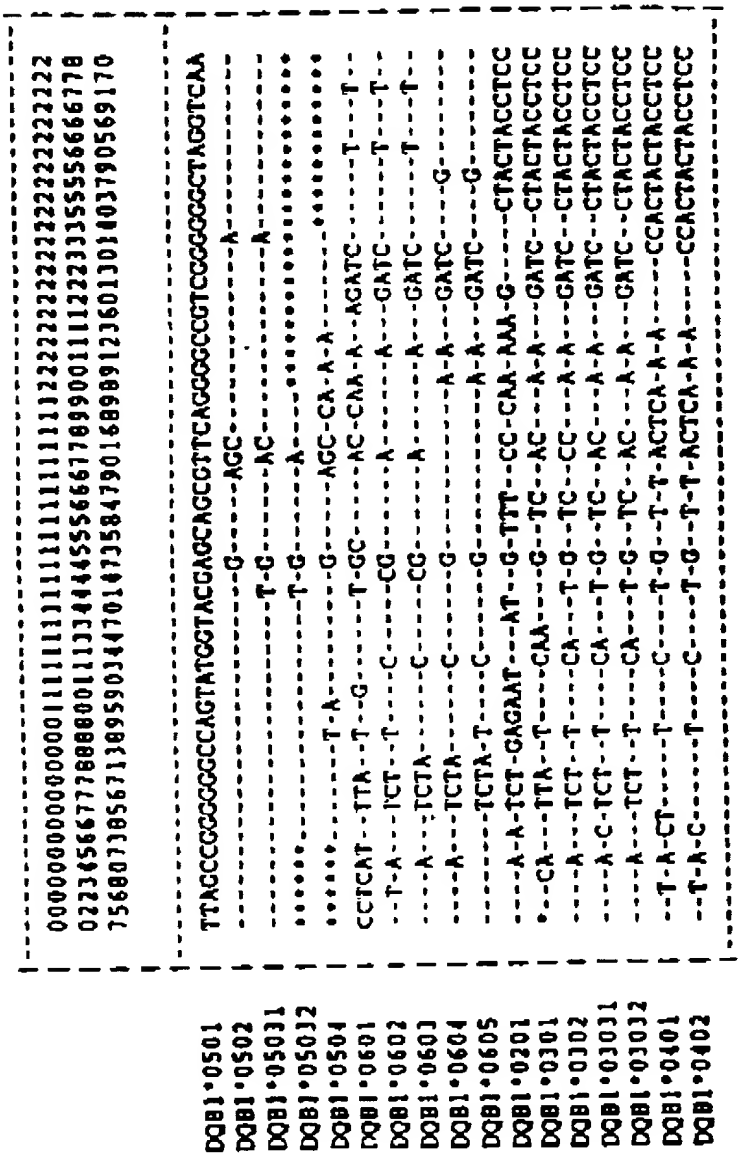
0000000000000011111111111111112222222222222222222222222222
0223456677788800111334445556667899001111222333555556666778
7568073856711895903447014735847901689891236013014037905691 70
TTACCCGGGGGCCAGTATGGTACGAGCAGCGTTCAGCGGCCGTCGGGGGCTAGGTCAA
DQB1*0501
DQB1*0502
DQB1*05031
DQB1*05032
DQB1*0504
DQB1*0601
DQB1*0602
DQB1*0603
DQB1*0604
DQB1*0605
DQB1*0201
DQB1*0301
DQB1*0302
DQB1*03031
DQB1*03032
DQB1*0401
DQB1*0402

FIGURE 9

FIGURE 10

Solution:

	63	68	170	7	76	88	171	
DQB1*0201	A	G	C	T	C	A	C	
DQB1*03031	C	G	A	T	C	T	C	
DQB1*0402	C	G	A	T	G	T	C	
DQB1*0401	C	T	A	T	G	T	C	
DQB1*0601	G	G	A	C	T	T	C	10.1
DQB1*0603	G	G	A	T	C	C	T	
DQB1*03032	G	G	A	T	C	T	C	
DQB1*0602	G	G	A	T	C	T	T	
DQB1*05031	G	G	A	T	G	C	C	
DQB1*05032	G	G	A	*	G	C	T	
DQB1*0301	G	G	A	*	T	T	C	
DQB1*0302	G	G	C	T	C	T	C	
DQB1*0502	G	G	G	T	G	C	C	
DQB1*0504	G	G	G	*	G	C	C	
DQB1*0604	G	G	T	T	C	C	T	
DQB1*0605	G	G	T	T	C	C	T	
DQB1*0501	G	G	T	T	G	C	T	

Solution:

	63	68	170	7	77	88	171	
DQB1*0201	A	G	C	T	T	A	C	
DQB1*0402	C	G	A	T	G	T	C	
DQB1*03031	C	G	A	T	T	T	C	
DQB1*0401	C	T	A	T	G	T	C	10.2
DQB1*0601	G	G	A	C	A	T	C	
DQB1*0301	G	G	A	*	A	T	C	
DQB1*05031	G	G	A	T	G	C	C	
DQB1*05032	G	G	A	*	G	C	T	
DQB1*0603	G	G	A	T	T	C	T	
DQB1*03032	G	G	A	T	T	T	C	
DQB1*0602	G	G	A	T	T	T	T	
DQB1*0302	G	G	C	T	T	T	C	
DQB1*0502	G	G	G	T	G	C	C	
DQB1*0504	G	G	G	*	G	T	C	
DQB1*0501	G	G	T	T	G	C	T	
DQB1*0604	G	G	T	T	T	C	T	
DQB1*0605	G	G	T	T	T	T	T	

Solution:

	63	68	170	26	76	88	171
DQB1*0201	A	G	C	A	C	A	C
DQB1*03031	C	G	A	A	C	T	C
DQB1*0402	C	G	A	T	G	T	C
DQB1*0401	C	T	A	T	G	T	C
DQB1*0603	C	G	A	A	C	C	T
DQB1*03032	C	G	A	A	C	T	C
DQB1*05031	C	G	A	A	G	C	C
DQB1*05032	C	G	A	*	G	C	T
DQB1*0301	G	G	A	A	T	T	C
DQB1*0602	C	G	A	T	C	T	T
DQB1*0601	G	G	A	T	T	T	C
DQB1*0302	G	G	C	A	C	T	C
DQB1*0502	C	G	G	A	G	C	C
DQB1*0504	C	G	G	*	G	T	C
DQB1*0604	C	G	T	A	C	C	T
DQB1*0605	C	G	T	A	C	T	T
DQB1*0501	C	G	T	A	G	C	T

10.3

Solution:

	63	68	170	26	76	88	231
DQB1*0201	A	G	C	A	C	A	G
DQB1*03031	C	G	A	A	C	T	G
DQB1*0402	C	G	A	T	G	T	C
DQB1*0401	C	T	A	T	G	T	C
DQB1*0603	G	G	A	A	C	C	G
DQB1*03032	G	G	A	A	C	T	G
DQB1*05031	G	G	A	A	G	C	A
DQB1*05032	G	G	A	*	G	C	*
DQB1*0301	G	G	A	A	T	T	G
DQB1*0602	G	G	A	T	C	T	G
DQB1*0601	G	G	A	T	T	T	G
DQB1*0302	G	G	C	A	C	T	G
DQB1*0502	G	G	G	A	G	C	A
DQB1*0504	C	G	G	*	G	T	G
DQB1*0604	C	G	T	A	C	C	G
DQB1*0605	G	G	T	A	C	T	G
DQB1*0501	G	G	T	A	G	C	G

10.4

Solution:

	63	68	170	26	77	88	231	
DQB1*0201	A	G	C	A	T	A	C	
DQB1*03031	C	G	A	A	T	T	C	
DQB1*0402	C	G	A	T	G	T	C	
DQB1*0401	C	T	A	T	G	T	C	
DQB1*0301	G	G	A	A	A	T	C	
DQB1*05031	G	G	A	A	G	C	C	
DQB1*05032	G	G	A	*	G	C	T	
DQB1*0603	G	G	A	A	T	C	T	10.5
DQB1*03032	G	G	A	A	T	T	C	
DQB1*0601	G	G	A	T	A	T	C	
DQB1*0602	G	G	A	T	T	T	C	
DQB1*0302	G	G	C	A	T	T	C	
DQB1*0502	G	G	G	A	G	C	C	
DQB1*0504	G	G	G	*	G	C	C	
DQB1*0501	G	G	T	A	G	C	T	
DQB1*0604	G	G	T	A	T	C	T	
DQB1*0605	G	G	T	A	T	T	T	

Solution:

	63	68	170	26	77	88	231	
DQB1*0201	A	G	C	A	T	A	G	
DQB1*03031	C	G	A	A	T	T	G	
DQB1*0402	C	G	A	T	G	T	C	
DQB1*0401	C	T	A	T	G	T	C	
DQB1*0301	G	G	A	A	A	T	G	
DQB1*05031	G	G	A	A	G	C	A	10.6
DQB1*05032	G	G	A	*	G	C	*	
DQB1*0603	G	G	A	A	T	C	G	
DQB1*03032	G	G	A	A	T	T	G	
DQB1*0601	G	G	A	T	A	T	G	
DQB1*0602	G	G	A	T	T	T	G	
DQB1*0302	G	G	C	A	T	T	G	
DQB1*0502	G	G	G	A	G	C	A-	
DQB1*0504	G	G	G	*	G	C	G	
DQB1*0501	G	G	T	A	G	C	G	
DQB1*0604	G	G	T	A	T	C	G	
DQB1*0605	G	G	T	A	T	T	G	

Solution:

	63	68	170	57	76	88	171	
DQB1*0201	A	G	C	C	C	A	C	
DQB1*03031	C	G	A	C	C	T	C	
DQB1*0402	C	G	A	C	G	T	C	10.7
DQB1*0401	C	T	A	C	G	T	C	
DQB1*0603	G	G	A	C	C	T	T	
DQB1*03032	G	G	A	C	C	T	C	
DQB1*0602	G	G	A	C	C	T	T	
DQB1*05031	G	G	A	C	G	C	C	
DQB1*05032	G	G	A	•	G	C	T	
DQB1*0301	G	G	A	C	T	T	C	
DQB1*0601	G	G	A	T	T	T	C	
DQB1*0302	G	G	C	C	C	T	C	
DQB1*0502	G	G	G	C	G	C	C	
DQB1*0504	G	G	G	•	G	T	C	
DQB1*0604	G	G	T	C	C	C	T	
DQB1*0605	G	G	T	C	C	T	T	
DQB1*0501	G	G	T	C	G	C	T	

Solution:

	63	68	170	57	77	88	171	
DQB1*0201	A	G	C	C	T	A	C	
DQB1*0402	C	G	A	C	G	T	C	
DQB1*03031	C	G	A	C	T	T	C	
DQB1*0401	C	T	A	C	G	T	C	
DQB1*0301	G	G	A	C	A	T	C	
DQB1*05031	G	G	A	C	G	C	C	
DQB1*05032	G	G	A	•	G	C	T	
DQB1*0603	G	G	A	C	T	C	T	10.8
DQB1*03032	G	G	A	C	T	T	C	
DQB1*0602	G	G	A	C	T	T	T	
DQB1*0601	G	G	A	T	A	T	C	
DQB1*0302	G	G	C	C	T	T	C	
DQB1*0502	G	G	G	C	G	C	C	
DQB1*0504	G	G	G	•	G	T	C	
DQB1*0501	G	G	T	C	G	C	T	
DQB1*0604	G	G	T	C	T	C	T	
DQB1*0605	G	G	T	C	T	T	T	

Solution:

	63	68	170	76	88	109	171	
DQB1*0201	A	G	C	C	A	A	C	
DQB1*03031	C	G	A	C	T	T	C	
DQB1*0402	C	G	A	G	T	T	C	
DQB1*0401	C	T	A	G	T	T	C	
DQB1*0603	G	G	A	C	C	T	T	
DQB1*03032	G	G	A	C	T	T	C	
DQB1*0602	G	G	A	C	T	T	T	
DQB1*05031	G	G	A	G	C	T	C	10.9
DQB1*05032	G	G	A	G	C	T	T	
DQB1*0601	G	G	A	T	T	G	C	
DQB1*0301	G	G	A	T	T	T	C	
DQB1*0302	G	G	C	C	T	T	C	
DQB1*0502	G	G	G	G	C	T	C	
DQB1*0504	G	G	G	G	T	T	C	
DQB1*0604	G	G	T	C	C	T	T	
DQB1*0605	G	G	T	C	T	T	T	
DQB1*0501	G	G	T	G	C	T	T	

Solution:

	63	68	170	76	88	113	171	
DQB1*0201	A	G	C	C	A	T	C	
DQB1*03031	C	G	A	C	T	C	C	
DQB1*0402	C	G	A	G	T	C	C	10.10
DQB1*0401	C	T	A	G	T	C	C	
DQB1*0603	G	G	A	C	C	C	T	
DQB1*03032	G	G	A	C	T	C	C	
DQB1*0602	G	G	A	C	T	C	T	
DQB1*05031	G	G	A	G	C	T	C	
DQB1*05032	G	G	A	G	C	T	T	
DQB1*0301	G	G	A	T	T	C	C	
DQB1*0601	G	G	A	T	T	T	C	
DQB1*0302	G	G	C	C	T	C	C	
DQB1*0502	G	G	G	G	C	T	C	
DQB1*0504	G	G	G	G	T	T	C	
DQB1*0604	G	G	T	C	C	C	T	
DQB1*0605	G	G	T	C	T	C	T	
DQB1*0501	G	G	T	G	C	T	T	

Solution:

	63	68	170	76	88	114	171
DQB1*0201	A	G	C	C	A	G	C
DQB1*03031	C	G	A	C	T	A	C
DQB1*0402	C	G	A	G	T	G	C
DQB1*0401	C	T	A	G	T	G	C
DQB1*0603	G	G	A	C	C	G	T
DQB1*03032	G	G	A	C	T	A	C
DQB1*0602	G	G	A	C	T	G	T
DQB1*05031	G	G	A	G	C	G	C
DQB1*05032	G	G	A	G	C	G	T
DQB1*0301	G	G	A	T	T	A	C
DQB1*0601	G	G	A	T	T	G	C
DQB1*0302	C	G	C	C	T	A	C
DQB1*0502	G	G	G	G	C	G	C
DQB1*0504	G	G	G	G	T	G	C
DQB1*0604	G	G	T	C	C	G	T
DQB1*0605	G	G	T	C	T	G	T
DQB1*0501	G	G	T	G	C	G	T

10.11

Solution:

	63	68	170	76	88	114	231
DQB1*0201	A	G	C	C	A	G	G
DQB1*03031	C	G	A	C	T	A	G
DQB1*0402	C	G	A	G	T	G	C
DQB1*0401	C	T	A	G	T	G	C
DQB1*0603	G	G	A	C	C	G	G
DQB1*03032	G	G	A	C	T	A	G
DQB1*0602	G	G	A	C	T	G	G
DQB1*05031	G	G	A	G	C	G	A
DQB1*05032	G	G	A	G	C	G	A
DQB1*0301	G	G	A	T	T	A	G
DQB1*0601	G	G	A	T	T	G	G
DQB1*0302	G	G	C	C	T	A	G
DQB1*0502	G	G	G	G	C	G	A
DQB1*0504	G	G	G	G	T	G	G
DQB1*0604	G	G	T	C	C	G	G
DQB1*0605	G	G	T	C	T	G	G
DQB1*0501	G	G	T	G	C	G	G

10.12

Solution:

	63	68	170	76	88	134	171
DQB1*0201	A	G	C	C	A	G	C
DQB1*03031	C	G	A	C	T	G	C
DQB1*0402	C	G	A	G	T	G	C
DQB1*0401	C	T	A	G	T	G	C
DQB1*0603	G	G	A	C	C	G	T
DQB1*03032	G	G	A	C	T	G	C
DQB1*0602	G	G	A	C	T	G	T
DQB1*05031	G	G	A	G	C	G	C
DQB1*05032	G	G	A	G	C	G	T
DQB1*0301	G	G	A	T	T	A	C
DQB1*0601	G	G	A	T	T	G	C
DQB1*0302	G	G	C	C	T	G	C
DQB1*0502	G	G	G	G	C	G	C
DQB1*0504	G	G	G	G	T	G	C
DQB1*0604	G	G	T	C	C	G	T
DQB1*0605	G	G	T	C	T	G	T
DQB1*0501	G	G	T	G	C	G	T

10.13

Solution:

	63	68	170	76	88	141	171
DQB1*0201	A	G	C	C	A	C	C
DQB1*03031	C	G	A	C	T	T	C
DQB1*0402	C	G	A	G	T	T	C
DQB1*0401	C	T	A	G	T	T	C
DQB1*0603	G	G	A	C	C	C	T
DQB1*0602	G	G	A	C	T	C	T
DQB1*03032	G	G	A	C	T	T	C
DQB1*05031	G	G	A	G	C	T	C
DQB1*05032	G	G	A	G	C	T	T
DQB1*0301	G	G	A	T	T	C	C
DQB1*0601	G	G	A	T	T	T	C
DQB1*0302	G	G	C	C	T	T	C
DQB1*0502	G	G	G	G	C	C	C
DQB1*0504	G	G	G	G	T	C	C
DQB1*0604	G	G	T	C	C	C	T
DQB1*0605	G	G	T	C	T	C	T
DQB1*0501	G	G	T	G	C	C	T

10.14

Solution:

	63	68	170	76	88	141	231
DQB1*0201	A	G	C	C	A	C	G
DQB1*03031	C	G	A	C	T	T	G
DQB1*0402	C	G	A	G	T	T	C
DQB1*0401	C	T	A	G	T	T	C
DQB1*0603	G	G	A	C	C	C	G
DQB1*0602	G	G	A	C	T	C	G
DQB1*03032	G	G	A	C	T	T	G
DQB1*05031	G	G	A	G	C	T	A
DQB1*05032	G	G	A	G	C	T	*
DQB1*0301	G	G	A	T	T	C	G
DQB1*0601	G	G	A	T	T	T	G
DQB1*0302	G	G	C	C	T	T	G
DQB1*0502	G	G	G	G	C	C	A
DQB1*0504	G	G	G	G	T	C	G
DQB1*0604	G	G	T	C	C	C	G
DQB1*0605	G	G	T	C	T	C	G
DQB1*0501	G	G	T	G	C	C	G

10.15

Solution:

	63	68	170	76	88	153	171
DQB1*0201	A	G	C	C	A	G	C
DQB1*03031	C	G	A	C	T	G	C
DQB1*0402	C	G	A	G	T	G	C
DQB1*0401	C	T	A	G	T	G	C
DQB1*0603	G	G	A	C	C	G	T
DQB1*03032	G	G	A	C	T	G	C
DQB1*0602	G	G	A	C	T	G	T
DQB1*05031	G	G	A	G	C	G	C
DQB1*05032	G	G	A	G	C	G	T
DQB1*0601	G	G	A	T	T	C	C
DQB1*0301	G	G	A	T	T	G	C
DQB1*0302	G	G	C	C	T	G	C
DQB1*0502	G	G	G	G	C	G	C
DQB1*0504	G	G	G	G	T	G	C
DQB1*0604	G	G	T	C	C	G	T
DQB1*0605	G	G	T	C	T	G	T
DQB1*0501	G	G	T	G	C	G	T

10.16

Solution:

	63	68	170	76	88	158	171	
DQB1*0201	A	G	C	C	A	T	C	
DQB1*03031	C	G	A	C	T	T	C	
DQB1*0402	C	G	A	G	T	T	C	
DQB1*0401	C	T	A	G	T	T	C	
DQB1*0603	G	G	A	C	C	A	T	
DQB1*0602	G	G	A	C	T	A	T	10.17
DQB1*03032	G	G	A	C	T	T	C	
DQB1*05031	G	G	A	G	C	A	C	
DQB1*05032	G	G	A	G	C	A	T	
DQB1*0601	G	G	A	T	T	A	C	
DQB1*0301	G	G	A	T	T	T	C	
DQB1*0302	G	G	C	C	T	T	C	
DQB1*0502	G	G	G	G	C	A	C	
DQB1*0504	G	G	G	G	T	A	C	
DQB1*0604	G	G	T	C	C	A	T	
DQB1*0605	G	G	T	C	T	A	T	
DQB1*0501	G	G	T	G	C	A	T	

Solution:

	63	68	170	76	88	158	231	
DQB1*0201	A	G	C	C	A	T	G	
DQB1*03031	C	G	A	C	T	T	G	
DQB1*0402	C	G	A	G	T	T	C	
DQB1*0401	C	T	A	G	T	T	C	
DQB1*0603	G	G	A	C	C	A	G	10.18
DQB1*0602	G	G	A	C	T	A	C	
DQB1*03032	G	G	A	C	T	T	G	
DQB1*05031	G	G	A	G	C	A	A	
DQB1*05032	G	G	A	G	C	A	*	
DQB1*0601	G	G	A	T	T	A	G	
DQB1*0301	G	G	A	T	T	T	G	
DQB1*0302	G	G	C	C	T	T	G	
DQB1*0502	G	G	G	G	C	A	A	
DQB1*0504	G	G	G	G	T	A	G	
DQB1*0604	G	G	T	C	C	A	G	
DQB1*0605	G	G	T	C	T	A	G	
DQB1*0501	G	G	T	G	C	A	G	

Solution:

	63	68	170	76	88	164	171	
DQB1*0201	A	G	C	C	A	T	C	
DQB1*03031	C	G	A	C	T	C	C	
DQB1*0402	C	G	A	G	T	G	C	
DQB1*0401	C	T	A	G	T	G	C	
DQB1*0603	G	G	A	C	C	G	T	
DQB1*03032	G	G	A	C	T	C	C	10.19
DQB1*0602	G	G	A	C	T	G	T	
DQB1*05031	G	G	A	G	C	G	C	
DQB1*05032	G	G	A	G	C	G	T	
DQB1*0301	G	G	A	T	T	C	C	
DQB1*0601	G	G	A	T	T	G	C	
DQB1*0302	G	G	C	C	T	C	C	
DQB1*0502	G	G	G	G	C	G	C	
DQB1*0504	G	G	G	G	T	G	C	
DQB1*0604	G	G	T	C	C	G	T	
DQB1*0605	G	G	T	C	T	G	T	
DQB1*0501	G	G	T	G	C	G	T	

Solution:

	63	68	170	76	88	164	231	
DQB1*0201	A	G	C	C	A	T	G	
DQB1*03031	C	G	A	C	T	C	G	
DQB1*0402	C	G	A	G	T	G	C	
DQB1*0401	C	T	A	G	T	G	C	10.20
DQB1*0603	G	G	A	C	C	G	G	
DQB1*03032	G	G	A	C	T	C	G	
DQB1*0602	G	G	A	C	T	G	G	
DQB1*05031	G	G	A	G	C	G	A	
DQB1*05032	G	G	A	G	C	G	*	
DQB1*0301	G	G	A	T	T	C	G	
DQB1*0601	G	G	A	T	T	G	G	
DQB1*0302	G	G	C	C	T	C	G	
DQB1*0502	G	G	G	G	C	G	A	
DQB1*0504	G	G	G	G	T	G	G	
DQB1*0604	G	G	T	C	C	G	G	
DQB1*0605	G	G	T	C	T	G	G	
DQB1*0501	G	G	T	G	C	G	G	